Concentrated Animal Feeding Operations as a Source of EDCs and their Management

Marc Mills[1], Steve Hutchins[1], James Lazorchak[2], Gary Ankley[3]
[1]U.S. Environmental Protection Agency, ORD/National Risk Management Research Laboratory, [2]U.S. Environmental Protection Agency, ORD/National Exposure Research Laboratory,[3]U.S. Environmental Protection Agency, ORD/National Health & Environmental Effects Research Laboratory

Science Questions

Problem/Issues: In the United States, there is an estimated 376,000 animal feed operations that confine animals, generating approximately 128 billion pounds of waste each year. A facility is an animal feed operation (AFO) if animals are stabled/confined, or fed/maintained, for 45 days or more within any 12-month period, and the facility does not produce any crops, vegetation, or forage growth. Concentrated animal feed operations (CAFOs) are the largest of these and are regulated under the Clean Water Act. CAFOs are generally considered to be operations with more than 1000 animal units. EDCs are known to be used or naturally produced by the 3 major categories of CAFOs - cattle, poultry, and swine. Cattle CAFOs use steroid hormones, including estrogens (estradiol, estradiol benzoate) and androgens (trenbolone acetate, testosterone propionate) in ear implants to promote growth. Poultry and swine CAFOs do not add steroid hormones per se, but the animals produce natural estrogens, estrogen conjugates, and testosterone.

Determining the Impact of EDCs on Humans, Wildlife, and the Environment

- How and to what degree are human and wildlife populations exposed to EDCs?
- What are the chemical classes of interest and their potencies?
- What are the major sources and environmental fates of EDCs?
- To what extent does CAFOs contribute estrogens to ground water?
- What is the fate of estrogens in ground water?

Research Goals

NRMRL, NHEERL and NERL have been developing analytical and biological tools to assess the extent, exposure and effects of these estrogens and androgens in ground and surface waters.

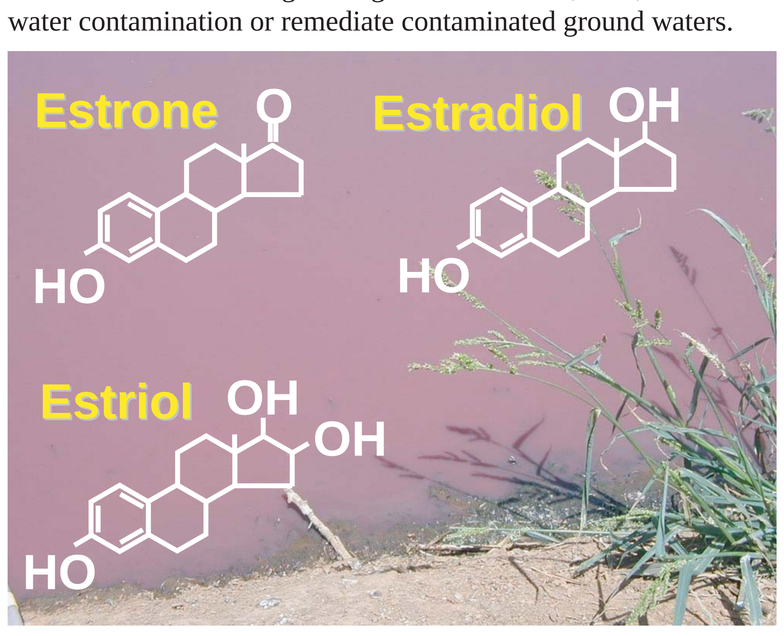
- For NRMRL, the focus is on the natural and synthetic estrogens and their potential for contamination of ground water following land application of CAFO waste. The research goals are to develop methods for analysis of estrogens and estrogen conjugates in complex matrices such as swine waste, and to use these methods to transport into the environment. Future research goals will be to use these and other methods to assess fate of estrogens in soils recieving waters and ground water and evaluate risk management strategies designed to minimize contamination events.

- The purpose of NERL's CAFO study was to evaluate the potential for the fathead minnow vitellogenin gene expression assay as an indicator of androgenic substances like 17α- and 17ß-trenbolone in a beef feedlot discharge, and in river water upstream and downstream from the discharge. NERL's objective was to see if sexually mature female fathead minnows (Pimephales promelas), would show reduced levels of vitellogenin gene expression when exposed to androgens.

- The goal of NHEERL's CAFO research was to: 1) assess androgenic activity and the presence of measured concentrations of α- and/or ß-trenbolone over time in discharge from a CAFO in Ohio, 2) develop a high quality data set concerning the effects of α- and ß-trenbolone on reproductive endocrinology and success using a model small fish species (the fathead minnow, Pimephales promelas), and 3) utilize field exposure and laboratory effects data to conduct preliminary assessment of the potential ecological risk of androgenic growth promoters in aquatic environments.

Methods/Approach (NRMRL)

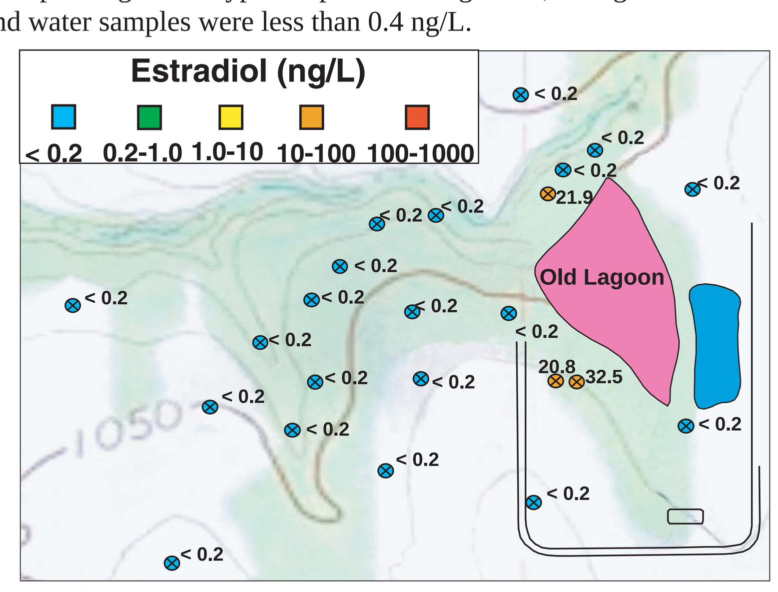
NRMRL has been working on developing GC/MS/MS and LC/MS/MS methods for analyzing swine lagoon effluent and ground water for estrogens and estrogen conjugates at environmentally relevant levels (ng/L). The primary research goal is to then use these methods to ascertain whether CAFOs contribute estrogens to ground water and, if so, to determine what risk management strategies will be most effective to either prevent ground water contamination or remediate contaminated ground waters.



Although all types of CAFOs are of interest, NRMRL's initial focus is on swine CAFOs. Swine lagoon waste is very complex and represents unique analytical challenges, especially since natural estrogens are potent EDCs and require analysis at parts-per-trillion (ng/L) levels. Also, swine excrete estrogens primarily in urine (96%), and urinary estrogens are most likely excreted in conjugate form.

NRMRL has developed a GC/MS/MS method suitable for analyzing swine lagoon waste and ground water for estrogens at ng/L levels, and has used this to measure estrogen levels in swine waste and ground water at three CAFO sites (1-2). For two sites, we find that swine lagoons contain 390-24900 ng/L estrone, 180-10400 ng/L estriol, and 40-3000 ng/L estradiol, with varying levels depending on the type of operation. In general, estrogen concentrations in ground water samples were less than 0.4 ng/L.

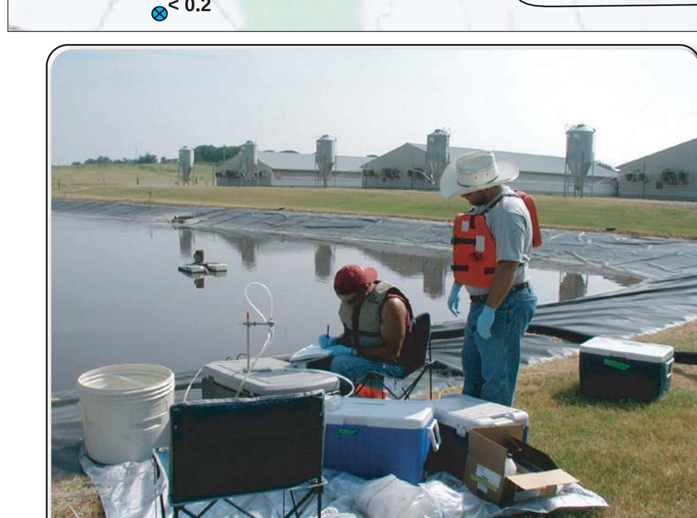
However, one site provided a notable exception – this was a closed facility which had a lagoon that had leaked into ground water, and measurable levels of all three estrogens were found in ground water adjacent to the lagoon. Example data for estradiol are shown here



We monitored these wells one year later and found that estrogen levels had generally increased, in some cases by an order of magnitude. Annual monitoring will continue so that we can evaluate the persistence of these estrogens in the ground water.

These initial studies focused on free estrogens, because we had not yet developed methods for analysis of estrogen conjugates. We have since found that swine lagoon waste can contain estrogens as both sulfate and glucuronide conjugates, and that these levels can be significant (eg, 107 ng/L free estradiol compared to > 2900 ng/L free + conjugated estradiol).

Why is this significant? Conjugated estrogens are expected to be more mobile in the environment because of their higher water solubility. There are 36 possible conjugates for these estrogens; of the 9 we evaluated, 3 were found, 3 were not, and 3 were indeterminate. Collectively, these data show that swine lagoons contain significant concentrations of natural environmental estrogens in both free and conjugated forms and can contaminate ground water. Additional work is needed to better define the extent of the problem, more fully address the estrogen conjugates issue, and



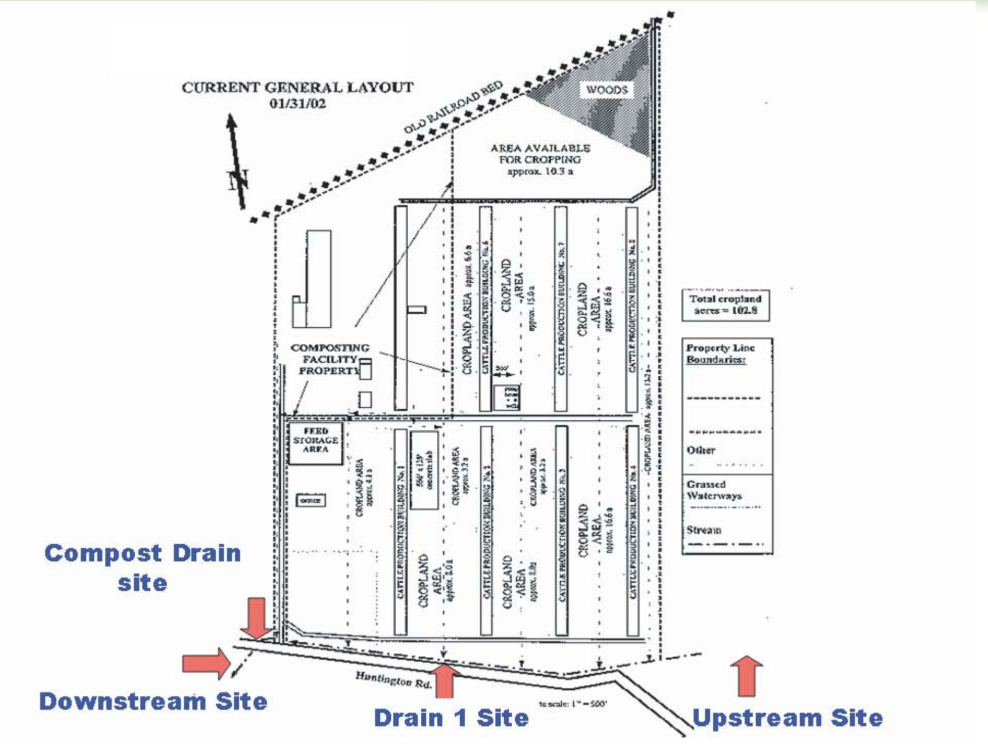
Methods/Approach (NERL)

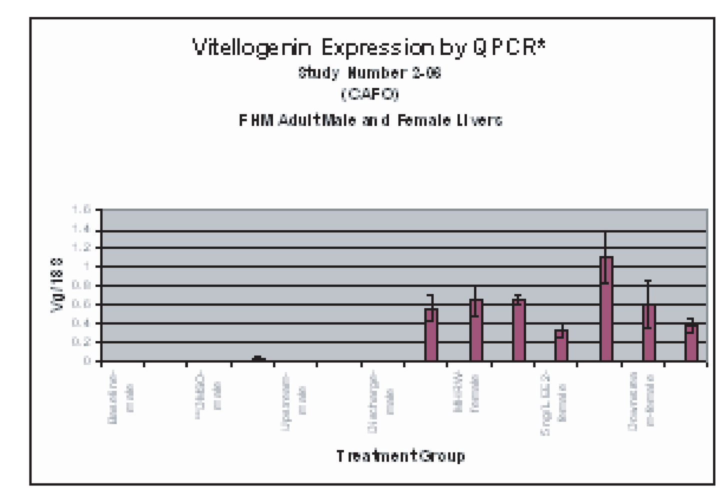
NERL has applied a vitellogenin gene expression assay to detect both pure trenbolone and trenbolone acetate in cattle CAFO discharges in Ohio and Iowa. The approach is to use down regulation of vitellogenin (vtg) gene expression in female fathead minnows as an indicator of androgens and the up regulation of vtg in males as indicators of estrogens. In addition, NERL is collaborating with USGS and the State of West Virginia to look at potential linkages of poultry wastes to high incidences of Ova-testis in male small mouth bass.

Study Site

?The CAFO used in this study was a beef facility located in Ohio near the Little Miami River. It was constructed in the mid-1960's and consists of eight cattle buildings on about 96 ha. In addition to the CAFO there are two additional agricultural associated industries, a compositing facility and a feed production facility. Both use manure wastes from the study CAFO. The waste is handled as a solid material with a typical moisture content of 40-50%. Shallow drains from the buildings collect ground water. During this study only one of these shallow drains showed evidence of flow. As of early 2002, the feeding operation had a capacity to hold and feed 9800 head of cattle. Revalor S7 implants, which contain both trenbolone acetate and 17ß-estradiol were used at this facility during the study.

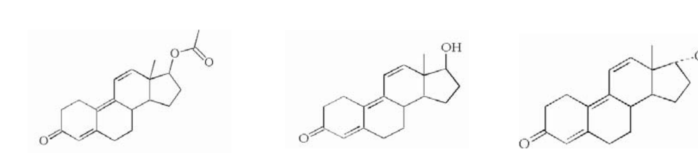
Four locations on the mainstream headwaters of the Little Miami River were chosen for sample collection : (1) upstream from all drainage from the facility, (2) a drain which collects run-off from the first two sets of buildings, (3) a drain from a compost facility and (4) downstream from all drains. Samples were collected on three different occasions, listed by sample identifier and date: February 2002, January 2003, and March 2003.





Methods/Approach (NHEERL)

NHEERL has developed an analytical chemistry method that detects the occurrence of 17a- and 17ß-trenbolone in cattle feedlot discharge and in river water (3) and has measured in vitro androgenic activity of the discharge using CV-1 cells that had been transiently cotransfected with human androgen receptor and reporter gene constructs (Vickie Wilson and Earl Gray in RTD (Reproduction and Toxicology Division, NHEERL, RTP).

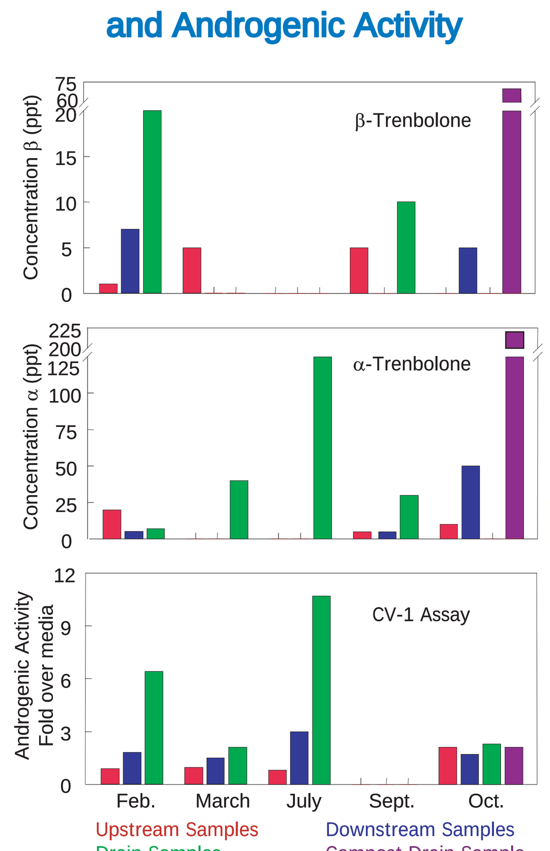


Trenbolone Acetate   17ß-Trenbolone   17α-Trenbolone

High Pressure Liquid Chromatography (HPLC) /Mass Spectrometry (MS)
Procedure: Samples were processed directly, or concentrated using C18 solid phase extraction, and analyzed on an Agilent 1100 HPLC with a fluorescence detector; excitation wavelength was 359 nm and emission was 458 nm. A gradient program with a mobile phase of methanol and water were used with a Nucleosil column. / Procedure: Trenbolone was measured un-derivatized by injecting the concentrated SPE extract onto a Delta-3 GC column (Machery-Nagel) and analyzed with a mass spectrometer (Agilent MSD) in the single ion monitoring (SIM) or full scan mode.

Determination of Androgenic Activity
Androgenic activity in water samples was determined using CV-1 cells (monkey kidney line, ATCC# CCL-70) transiently cotransfected with an human androgen receptor vector (pCMVhAR) and MMTV (mouse mammary tumor virus)-luciferase reporter using Fugene reagent. Twenty-four h after transfection, the original DMEM medium was removed from the cells and replaced with medium that had been prepared from dried materials with site water that had been passed through a 0.2 micron nylon filter. After 24 h, cells were harvested, and relative light intensity determined using a luminometer. CV-1 assays with each test fraction were performed, and a DHT positive control was included with each set of analyses.

HPLC Determined Trenbolone Concentrations and Androgenic Activity



Results/Conclusions

Collectively, ORD's research has shown that CAFOs do have the potential for release of hormones as EDCs into the environment:

- NRMRL's research shows that ground water can become contaminated with estrogens from swine operations, and that estrogen conjugates may comprise a significant portion of the overall estrogen load.

- NERL's vitellogenin assay results have not shown appreciable reductions in vitellogenin gene expression when exposed to either CAFO samples containing trenbolone concentrations nor positive trenbolone control samples. Reasons for this can be that the duration of exposure was not long enough and/or trenbolone degrades or is rapidly absorbed and the exposure concentration is much less than nominal and cannot be determined. NERL has developed a constant addition method that should take care of the second potential problem and will be testing it shortly.

Impact and Outcomes

ORD's research in this area is only just beginning and has been devoted to developing the tools necessary to assess the risk that CAFOs pose for the release of hormones into the environment. This process is ongoing and must continue in order to provide sufficient information for EPA to decide:

- is risk too great based on environmental data?
- do more sites need to be studied?
- can we make recommendations for land management strategies based on what we have?
- can we use these tools to assess effectiveness of new land management strategies?

The ultimate impact of this research will be to determine whether CAFOs provide sufficient risk so as to require regulation or management to minimize release of EDCs.

Future Directions

Currently, ORD is developing analytical and biological approaches to measure and detect EDCs in ground and surface waters and assess the potential exposures to aquatic life. A collaborative effort will be initiated across ORD laboratories to select field sites where these tools can be used collectively to further assess the potential exposure and effects of EDCs from CAFOs on ground water and surface water communities. This information will then be provided to the Regions and program offices for decisions on best management practices for controlling EDCs.

Products

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